

a) Amendment to the Claims

Claims 1-3 (Cancelled).

4. (Currently Amended) A process for producing N-acetylneuraminic acid which comprises:

~~providing providing, in an aqueous medium (i) a culture of a microorganism having N-acetylneuraminic acid aldolase activity or N-acetylneuraminic acid synthetase activity, or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells; ; (ii) a culture of a microorganism capable of producing pyruvic acid or a treated matter of the culture selected from the group consisting of the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells when a culture of a microorganism having N-~~  
~~acetylneuraminic acid aldolase activity is used in (i) above, or a culture of a microorganism capable of producing phosphoenolpyruvic acid or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization,~~

and an enzyme preparation obtained by extracting the cells when a culture of a microorganism having N-acetylneuraminic acid synthetase activity is used in (i) above; (iii) N-acetylmannosamine which is produced by allowing a culture of a microorganism harboring DNA encoding N-acetylglucosamine 2-epimerase isolated obtained from a microorganism belonging to the genus *Synechocystis*, or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells and N-acetylglucosamine to be present in an the aqueous medium to form and accumulate N-acetylmannosamine in the culture medium, and (iv) an energy source which is necessary for the formation of a pyruvic acid or phosphoenolpyruvic acid;

allowing N-acetylneuraminic acid to form and accumulate in the aqueous medium; and

recovering N-acetylneuraminic acid from the aqueous medium  
wherein said cultures are independently provided as cultures per se  
or treated matters thereof, wherein said treated matters are selected from the group  
consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture,  
products obtained by treating the cells by drying, freeze-drying, ultrasonication, mechanical  
friction, protein fractionation and cell-immobilization, and an enzyme preparation obtained  
by extracting the cells, or a preparation obtained by treatment with enzymes, surfactant or  
solvent, and wherein said treated matter continues to have said enzyme activity.

5. (Currently Amended) A process; process for producing N-acetylneuraminic acid which comprises:

providing providing, in aqueous medium (i) a culture of a microorganism having N-acetylneuraminic acid aldolase activity or N-acetylneuraminic acid synthetase activity, ~~or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells;~~ ; (ii) a culture of a microorganism capable of producing pyruvic acid or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, a treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells when a culture of a microorganism having N-acetylneuraminic acid aldolase activity is used in (i) above, or a culture of a microorganism capable of producing phosphoenolpyruvic acid ~~or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells when a culture of a microorganism having N-~~ acetylneuraminic acid synthetase activity is used in (i) above; ; (iii) N-acetylmannosamine which is produced by allowing a culture of a microorganism harboring DNA comprising SEQ ID NO:2 encoding N-acetylglucosamine 2-epimerase comprising SEQ ID NO:1 and being capable of forming N-acetylmannosamine from ~~or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by~~

centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction; treatment with a solvent, enzymatic treatment, protein fractionation and immobilization; and an enzyme preparation obtained by extracting the cells and N-acetylglucosamine to be present in an aqueous medium so as to form and accumulate N-acetylmannosamine in the aqueous medium, wherein said DNA encoding N-acetylglucosamine 2-epimerase is selected from the group consisting of: (a) DNA encoding a protein having the amino acid sequence shown in SEQ ID NO: 1; and (b) DNA having the nucleotide sequence shown in SEQ ID NO: 2; and (iv) an energy source which is necessary for the formation of pyruvic acid or phosphoenolpyruvic acid;

allowing N-acetylneuraminic acid to form and accumulate in the aqueous medium; and

recovering N-acetylneuraminic acid from the aqueous medium  
wherein said cultures are independently provided as cultures *per se*  
or treated matters thereof, wherein said treated matters are selected from the group  
consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture,  
products obtained by treating the cells by drying, freeze-drying, ultrasonication, mechanical  
friction, protein fractionation and cell-immobilization, and an enzyme preparation obtained  
by extracting the cells, or a preparation obtained by treatment with enzymes, surfactant or  
solvent, and wherein said treated matter continues to have said enzyme activity.

6. (Previously Presented) The process according to any one of claims 4, 5, 17 and 19, wherein said microorganism having N-acetylneuraminic acid aldolase activity is a microorganism belonging to the genus Escherichia or Corynebacterium.

7. (Previously Presented) The process according to any one of claims 4, 5, 18 and 20, wherein said microorganism having N-acetylneuraminic acid synthetase activity is a microorganism belonging to a genus selected from the group consisting of Escherichia, Neisseria and Streptococcus.

8. (Previously Presented) The process according to any one of claims 4, 5, 17 and 19, wherein said microorganism capable of producing pyruvic acid is a microorganism belonging to a genus selected from the group consisting of Escherichia, Corynebacterium and Saccharomyces.

9. (Previously Presented) The process according to any one of claims 4, 5, 18 and 20, wherein said microorganism capable of producing phosphoenolpyruvic acid is a microorganism belonging to a genus selected from the group consisting of Escherichia, Corynebacterium and Saccharomyces.

10. (Previously Presented) The process according to claim 6, wherein said microorganism belonging to the genus Escherichia is Escherichia coli.

11. (Previously Presented) The process according to claim 6, wherein said microorganism belonging to the genus Corynebacterium is Corynebacterium ammoniagenes, Corynebacterium glutamicum or Corynebacterium acetoacidophilum.

12. (Previously Presented) The process according to claim 7, wherein said microorganism belonging to the genus Escherichia is Escherichia coli.

13. (Previously Presented) The process according to claim 8, wherein said microorganism belonging to the genus Escherichia is Escherichia coli.

14. (Previously Presented) The process according to claim 9, wherein said microorganism belonging to the genus Escherichia is Escherichia coli.

15. (Previously Presented) The process according to claim 8, wherein said microorganism belonging to the genus Corynebacterium is Corynebacterium ammoniagenes, Corynebacterium glutamicum or Corynebacterium acetoacidophilum.

16. (Previously Presented) The process according to claim 9, wherein said microorganism belonging to the genus Corynebacterium is Corynebacterium ammoniagenes, Corynebacterium glutamicum or Corynebacterium acetoacidophilum.

17. (Currently Amended) A process for producing N-acetylneuraminic acid which comprises:

~~providing~~ providing in an aqueous medium (i) a culture of a microorganism having N-acetylneuraminic acid adolase activity; or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells; (ii) a culture of a microorganism capable of producing pyruvic acid; acid or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various

means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells, (iii) N-acetylmannosamine which is produced by allowing a culture of a microorganism carrying harboring DNA encoding N-acetylglucosamine 2-epimerase activity selected from the group consisting of (a) DNA encoding a protein having comprising the amino acid sequence shown in SEQ ID NO: 1 and being capable of forming N-acetylmannosamine from N-acetylglucosamine; and (b) DNA having comprising the nucleotide sequence shown in SEQ ID NO: 2 encoding N-acetylglucosamine 2-epimerase comprising SEQ ID NO:1 and being capable of forming N-acetylmannosamine from N-acetylglucosamine or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells, and N-acetylglucosamine to be present in an aqueous medium so as to form and accumulate N-acetylmannosamine in the aqueous medium; medium; and (iv) an energy source which is necessary for the formation of pyruvic acid;

allowing N-acetylneuraminic acid to form and accumulate in the aqueous medium; and

recovering N-acetylneuraminic acid from the aqueous medium  
wherein said cultures are independently provided as cultures *per se*  
or treated matters thereof, wherein said treated matters are selected from the group  
consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture,  
products obtained by treating the cells by drying, freeze-drying, ultrasonication, mechanical  
friction, protein fractionation and cell-immobilization, and an enzyme preparation obtained

by extracting the cells, or a preparation obtained by treatment with enzymes, surfactant or solvent, and wherein said treated matter continues to have said enzyme activity.

18. (Currently Amended) A process for producing N-acetylneuraminic acid which comprises:

providing providing, in an aqueous medium (i) a culture of a microorganism having N-acetylneuraminic acid synthetase activity; activity or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells; (ii) a culture of a microorganism capable of producing phosphoenolpyruvic acid; acid or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells; (iii) N-acetylmannosamine which is produced by allowing a culture of a microorganism carrying DNA encoding N-acetylglucosamine 2-epimerase activity harboring DNA selected from the group consisting of (a) DNA encoding a protein having comprising the amino acid sequence shown in SEQ ID NO: 1 and being capable of forming N-acetylmannosamine from N-acetylglucosamine; and (b) DNA having comprising the nucleotide sequence shown in SEQ ID NO: 2 encoding N-acetylglucosamine 2-epimerase comprising SEQ ID NO:1 and being capable of forming N-acetylmannosamine from N-acetylglucosamine or a treated matter of the culture selected from the group consisting of

concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells and N-acetylglucosamine to be present in an aqueous medium so as to form and accumulate N-acetylmannosamine in the aqueous medium; medium; and (iv) an energy source which is necessary for the formation of phosphoenolpyruvic acid;

allowing N-acetylneuraminic acid to form and accumulate in the aqueous medium; and

recovering N-acetylneuraminic acid from the aqueous medium

wherein said cultures are independently provided as cultures *per se*

or treated matters thereof, wherein said treated matters are selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by drying, freeze-drying, ultrasonication, mechanical friction, protein fractionation and cell-immobilization, and an enzyme preparation obtained by extracting the cells, or a preparation obtained by treatment with enzymes, surfactant or solvent, and wherein said treated matter continues to have said enzyme activity.

19. (Currently Amended) A process for producing N-acetylneuraminic acid which comprises:

providing providing, in an aqueous medium (i) a culture of a microorganism having N-acetylneuraminic acid adolase activity; activity or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation

and immobilization, an enzyme preparation obtained by extracting the cells; (ii) a culture of a microorganism capable of producing pyruvic acid; acid or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells; (iii) a culture of a microorganism having harboring DNA encoding N-acetylglucosamine 2-epimerase activity which carries DNA encoding N-acetylglucosamine 2-epimerase obtained isolated from a microorganism belonging to the genus *Synechocystis*, and *Synechocystis*; (iv) an energy source which is necessary for the formation of pyruvic acid; and (v) N-acetylglucosamine;

allowing N-acetylneuraminic acid to form and accumulate in the aqueous medium; and

recovering N-acetylneuraminic acid from the aqueous medium  
wherein said cultures are independently provided as cultures per se  
or treated matters thereof, wherein said treated matters are selected from the group  
consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture,  
products obtained by treating the cells by drying, freeze-drying, ultrasonication, mechanical  
friction, protein fractionation and cell-immobilization, and an enzyme preparation obtained  
by extracting the cells, or a preparation obtained by treatment with enzymes, surfactant or  
solvent, and wherein said treated matter continues to have said enzyme activity.

20. (Currently Amended) A process for producing N-acetylneuraminic acid which comprises:

providing providing, in an aqueous medium (i) a culture of a microorganism having N-acetylneuraminic acid synthetase activity; activity or a treated

matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells; (ii) a culture of a microorganism capable of producing phosphoenolpyruvic acid; acid or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells; (iii) a culture of a microorganism having harboring DNA encoding N-acetylglucosamine 2-epimerase activity which carries a DNA encoding N-acetylglucosamine 2-epimerase obtained isolated from a microorganism belonging to the genus Synechocystis, and Synechocystis; (iv) an energy source which is necessary for the formation of phosphoenolpyruvic acid; and (v) N-acetylglucosamine;

allowing N-acetylneuraminic acid to form and accumulate in the aqueous medium; and

recovering N-acetylneuraminic acid from the aqueous medium  
wherein said cultures are independently provided as cultures per se  
or treated matters thereof, wherein said treated matters are selected from the group  
consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture,  
products obtained by treating the cells by drying, freeze-drying, ultrasonication, mechanical  
friction, protein fractionation and cell-immobilization, and an enzyme preparation obtained  
by extracting the cells, or a preparation obtained by treatment with enzymes, surfactant or  
solvent, and wherein said treated matter continues to have said enzyme activity.

21. (Currently Amended) The process according to claim 17 or 19,  
wherein said culture of a microorganism ~~carrying DNA encoding N-acetylglucosamine 2-~~  
~~epimerase activity or treated matter thereof harboring DNA encoding N-acetylglucosamine~~  
~~2-epimerase~~ is copresent with said cultures of a ~~microogramism~~ microorganism having N-  
acetylneuraminic acid aldolase activity and said culture of microorganism capable of  
producing pyruvic acid ~~or treated matter thereof~~.

22. (Currently Amended) The process according to claim 18 or 20,  
wherein said culture of a microorganism ~~carrying DNA encoding N-acetylglucosamine 2-~~  
~~epimerase activity or treated matter thereof harboring DNA encoding N-acetylglucosamine~~  
~~2-epimerase~~ is copresent with said cultures of a ~~microogramism~~ microorganism having N-  
acetylneuraminic acid synthetase activity and said culture of microorganism capable of  
producing phosphoenolpyruvic acid ~~or treated matter thereof~~.